CLAIMS

That which is claimed is:

1. A method of preparing a gene vector, said method comprising:

a) transforming yeast cells with a RKO clone and a yeast targeting cassette (YTC), wherein said RKO clone comprises a genomic clone insert, a yeast replication element, a yeast selectable marker, a bacterial origin of replication, optionally a bacterial selectable marker, and optionally a mammalian negative selection marker, and wherein said YTC comprises a bacterial/mammalian positive selection marker flanked by recombinogenic arms;

b) maintaining said yeast cells under conditions wherein said RKO clone and said YTC undergo homologous recombination via said genomic clone insert and said recombinogenic arms to produce a gene targeting vector;

c) selecting transformed yeast cells by their expression of said yeast selectable marker on said gene targeting vector or on said RKO clone;

d) isolating said gene targeting vector and said RKO clone from said selected yeast cells;

e) transforming bacterial cells with said gene targeting vector and said RKO clone;

f) selecting transformed bacterial cells that grow on selective media that is selective for bacterial cells expressing said bacterial/mammalian positive selection marker, thereby selecting for bacterial cells transformed with said gene targeting vector; and

g) isolating said gene targeting vector from said selected bacterial cells.

2. The method of claim 1 wherein said bacterial cells are Escherichia coli.

3. The method of claim 1 wherein said RKO clone is a cosmid and further comprises at least 1 Cos site.

4. The method of claim 1 wherein said RKO clone further comprises a multiple cloning site, and wherein said genomic clone insert is present within said multiple cloning site.

5. The method of claim 1 wherein said YTC further comprises loxP or FRT sites flanking said mammalian positive selection marker.

6. The method of claim 1 wherein said RKO clone comprises a mammalian negative selection marker.

7. The method of claim 1 wherein said YTC is generated by a PCR reaction using chimeric oligonucleotides bearing sequence identity to both the bacterial/mammalian positive selection marker and the GRI.

8. The method of claim 1 wherein said YTC comprises an internal ribosomal entry site (IRES) element that allows protein translation of said bacterial/mammalian positive selection marker in mammalian cells to occur from mRNA transcripts driven by a promoter in the GRI.

9. The method of claim 1 wherein said bacterial/mammalian positive selection marker lacks a polyadenylation site on the 3' end thereof.

10. A method of preparing gene targeted mammalian cells having a targeted gene mutation, said method comprising:

a) transforming mammalian cells with said gene targeting vector of claim 1;

b) maintaining said mammalian cells under conditions wherein said gene targeting vector and the genome of said mammalian cells undergo homologous recombination to produce a gene targeted mammalian cell; and

c) selecting gene targeted mammalian cells wherein homologous recombination has occurred by selecting gene targeted mammalian cells for their expression of said bacterial/mammalian positive selection marker, thereby obtaining gene targeted mammalian cells containing said targeted gene mutation.

11. The method of claim 10 wherein said mammalian cells are stem cells.

12. The method of claim 10 wherein said mammalian cells are embryonic stem cells.

13. The method of claim 10 wherein said RKO clone comprises a mammalian negative selection marker, and said gene targeted mammalian cells are selected for their expression of said bacterial/mammalian positive selection marker and by their non-expression of said mammalian negative selection marker.

14. A method of making gene targeted mice, said method comprising:

a) combining a gene targeted mouse cell according to claim 11 with an early mouse embryo to produce a gene targeted embryonic construct, and

b) introducing said gene targeted embryonic construct into a female host mouse, wherein said gene targeted embryonic construct is allowed to mature into a chimeric live whole mouse, said whole mouse thereby having a genome that includes said targeted gene mutation.

15. A method of making homozygous gene targeted mice, said method comprising cross-breeding male and female mice obtained by the method of claim 14 to produce offspring mice, and selecting offspring mice from said cross-breeding that are homozygous for said targeted gene mutation.

16. A gene targeting vector comprising a yeast replication element, a yeast selectable marker, a bacterial origin of replication, optionally a bacterial selectable marker, optionally a mammalian negative selection marker, and agenomic clone insert containing a bacterial/mammalian positive selection marker inserted therein.

17. The gene targeting vector of claim 16 wherein said gene targeting vector comprises a mammalian negative selection marker.

18. The gene targeting vector of claim 16 further comprising at least 1 Cos site.

19. The gene targeting vector of claim 16 further comprising a multiple cloning site, and wherein said genomic clone insert is present within said multiple cloning site.

20. The gene targeting vector of claim 16 further comprising loxP or FRT sites flanking said mammalian positive selection marker.